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PII: S1618-8667(15)00093-X
DOI: http://dx.doi.org/doi:10.1016/j.ufug.2015.06.011
Reference: UFUG 25563

To appear in:

Received date: 18-9-2014
Revised date: 16-4-2015
Accepted date: 25-6-2015

Please cite this article as: Fini, A., Frangi, P., Faoro, M., Piatti, R., Amoroso, G., Ferrini, F.,Effects of different pruning methods on an urban tree species: a four-year-experiment scaling down from the whole tree to the chloroplasts, *Urban Forestry and Urban Greening* (2015), http://dx.doi.org/10.1016/j.ufug.2015.06.011

Effects of different pruning methods on an urban tree species: a four-year-experiment scaling down from the whole tree to the chloroplasts

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Abstract

The aim of this work was to evaluate the effects of repeated pruning interventions using different pruning methods on growth and physiology of *Acer pseudoplatanus* L. Trees were pruned in 2008 and 2010 according to widely used pruning techniques for urban trees, such as reduction cut, removal cut and heading (topping) cut. Crown dieback, growth of the plant and of the pruned branches, leaf morphological traits and leaf gas exchange were assessed during the two growing season after each pruning cycle. Topping cut (i.e. the pruning treatment which suppressed the primary axis without providing a substitute) induced changes on tree growth pattern (i.e. by increasing the release of adventitious watersprouts and root suckers and decreasing stem diameter growth), which were not observed in the other pruning treatments. At the leaf level only topping cut increased leaf area at the expense of leaf mass per area, which may contribute to explain the higher occurrence of dieback on topped branches than in control and in the other pruning treatments. Also, leaves on topped branches displayed higher chlorophyll content and higher activity of Calvin cycle enzymes, which did not translate in higher CO_2 assimilation. We show here that pruning method, not only its severity

(i.e. the amount of leaf area removed), modulates the morpho-physiological response of trees to pruning and that maintenance of apical control and apical dominance are key issues to preserve a structurally sound tree structure, as well as the long-term efficiency of the photosynthetic apparatus.

Key words: *Acer pseudoplatanus*, apical control, leaf gas exchange, reduction cut, removal cut, topping cut

Introduction

Trees growing in the urban environment require periodic pruning to provide clearance and improve view (i.e. trees along roadsides), to reduce conflicts with buildings and infrastructures, to thin dense canopies and decrease wind resistance, and to reduce risk of failure by removing structural defects (Dureya et al., 1996; Smiley and Kane, 2006).

Three types of pruning cuts are commonly used to prune urban trees (American National Standard Institute, 2008; Gilman, 2012): removal cut, reduction cut and heading (topping) cut. Removal cut removes the whole target branch at its attachment to the trunk or parent branch, thus eliminating the entire lateral growing axis. Reduction cut shortens the primary axis by removing the distal end to a smaller lateral branch, which should be at least one-third to one-half of the diameter of the removed portion and should assume the apical role for the remaining branch (Harris et al., 2004; Grabosky and Gilman, 2007). Finally, topping cut shortens the primary axis by cutting the distal portion of the branch in the internode or in between consecutive lateral branches. In this case, no properly-sized lateral is retained to assume the role of apical growing axis for the remaining branch (Harris et al., 2004). These pruning methods differ in the way the target branch and its apical portion are managed. It is long known that apical buds (of the tree main stem and of individual branches) control key physiological processes determining tree structure and growing pattern (Cline 1994, 1996). These include apical dominance (i.e. the inhibition of lateral bud sprouting by the apex in an individual branch) and apical control (i.e. the influence of apical growing axis on elongation and orientation of lateral shoots within an individual branch) (Martin, 1987; Cline, 1997). Much of research on pruning of urban trees, however, has focused on pruning severity and timing (Mierowska et al., 2002; Gilman and

Grabosky, 2009; Fini et al., 2013), on tree response to wounding (Solomon and Blum, 1977; Neely, 1979; Schwarze, 2008), on compartimentalization of wood decay fungi (Shigo and Marx, 1977; Schwarze, 2001; O'Hara, 2007; Schwarze et al., 2007) or on tree response in the wind (Gilman et al., 2008a, 2008b; Pavlis et al., 2008), whereas very little research has focused on the effects of pruning method on the long-term structure and physiology of urban trees (Clark and Matheny, 2010). Because of the lack of knowledge about the long-term physiological effects of pruning, it is not possible to set national and international standards and best practices aimed at improving tree health and longevity and, in several countries, pruning prescriptions are mostly based on operational needs and short-term cost criteria (Campanella et al., 2009; Maurin and DesRochers, 2013).

Most of the research investigating physiological and growth response to pruning has been conducted on fruit or timber trees (Lebon et al., 2004; Spann et al., 2008; Fumey et al., 2011; Maurin and DesRochers, 2013), but these findings may not be directly transferred to urban trees because pruning aims are completely different [i.e. improving fruit yield or quality and producing clearwood for fruit and timber production, respectively, while urban arboriculture is primary targeted to obtain large, healthy, long-lived trees with a sound structure, capable of providing large benefits to the community, see Nowak et al. (2002)]. Research on fruit and timber plantations showed that pruning stimulates emission of new sprouts from latent and adventitious buds on the pruned branch, depresses plant height and stem diameter growth, and depletes non-structural carbohydrates pool (Davidson and Remhprey, 1994; Spann et al., 2008), but the implications of these morphological changes to long-term structural soundness were beyond the aims of these works. Photosynthesis is also affected by pruning, often showing temporary increases (the so called "compensatory photosynthesis"), the extent of this increase being usually related to the amount of leaf area removed (Pinkard and Beadle, 1998; Medhurst et al., 2006). Whether the increase in photosynthesis is related to increased leaf nutrients and chlorophyll, to higher carboxylation efficiency and ribulose regeneration, to higher stomatal conductance, to the depletion of nonstructural carbohydrates pool or to altered source:sink ratio is still a matter of debate (Pinkard et al., 1998; Lavigne et al., 2001; Li et al., 2002; Turnbull et al., 2007).

The aim of this work was to evaluate the long-term effects of different pruning methods on the structure of the whole tree and of the pruned branches, as well as the effects on selected leaf traits and leaf gas exchange. We hypothesized that pruning method, not only its severity (i.e. the amount of leaf area removed, see Pinkard and Beadle, 1998; Medhurst et al., 2006), can modulate tree response, and that greater reaction to pruning by the tree may occur in treatments which mostly suppress apical control and dominance. In detail, we tested the following hypotheses: 1) topping cut may completely impair apical control and dominance, thereby promoting release of lateral sprouts from latent or adventitious buds and increasing the occurrence of codominant branching on the pruned branches; 2) reduction cut may, instead, preserve apical dominance and control, thereby resulting in much lower disturbance to tree structure; 3) the removal of the whole branch to its attachment to the trunk will provide minimal disturbance to tree morphological and physiological processes, because regrowth may be avoided by apical dominance exerted by the trunk; 4) all pruning treatments will induce similar increases in leaf biochemical parameters and photosynthetic rate, but competition among codominant sprouts will result, in the long-term, in greater decline of photosynthesis in topped trees; 5) pruning effects on trees will increase as pruning is repeated over time.

Materials and Methods

Plant material and environmental conditions

In spring 2005, 28 uniform 3.2-3.8 cm diameter (10-12 cm circumference) sycamore maples (*Acer pseudoplatanus* L.) were planted in an experimental plot at the Fondazione Minoprio (Vertemate con Minoprio, Como, Italy; 45°44’ N, 9°04’ E), in a loamy sand, well drained soil. Mean annual rainfall in the experimental site, calculated over the last 20 years, is 1086 mm and average temperature 12.3 °C. Daily temperature and rainfall were recorded using a weather station (Vantage Pro 2, Davis, San Francisco, CA, U.S.) throughout the experimental period (monthly average temperature and total rainfall are reported in Fig. 1). Mean yearly rainfall recorded during the experiment was greatly above the 20-year average except for 2011 (867 mm total rainfall), whereas mean yearly temperature was close to the 20-year average throughout the experiment.

Pruning treatments and experimental set up

After planting, trees were allowed to establish and grow undisturbed for 3 years. In February 2008 (1st pruning cycle), plants were pruned using bypass hand pruners, according to the following treatments (illustrated in Fig. 2): 1) Topping cut: pruning cuts were made in the middle of the internode of first-order lateral branches (over 3-year old); 2) Removal cut: first-order lateral branches (over 3-year old) were cut at their union with the stem, using care not to damage branch collar (Shigo, 1990); 3) Reduction cut: first-order lateral branches (over 3-year old) were cut back to a lateral with sufficient size to become a new leader. Therefore, all new leaders chosen had aspect ratio (calculated as ratio between the diameter of lateral chosen as new leader and that of the parent branch to be removed, both measured above the branch union) greater than 0.33 (Gilman, 2012); 4) Control: plants were left unpruned. In February 2010 (2nd pruning cycle), trees were pruned again according to the same treatments as in 2008. All cuts were made at nodes or internodes which were over 2-year old. Following the recommendations by ANSI A300 (American National Standard Institute, 2008), regardless of pruning method, pruning was carried out in order to reduce leaf area by 30%, which corresponds to a mild defoliation (Willard and McKell, 1978; Simard et al., 2012). Because trees were pruned during the dormant season, branch cross sectional area was used to estimate the amount of leaf area removed (Grabosky et al., 2007; Gilman and Grabosky, 2009). Also, while pruning, pruned material was weighed in order to confirm the removal of a similar amount of woody biomass in all pruning treatments. The weight of the pruned material was 1438±355 g and 2088±492 g, in the first and in the second pruning cycles, respectively, and was not affected by pruning method ($P = 0.333$ and 0.393 in the first and in the second pruning cycles, respectively). To remove the same amount of wood, removal cut required about 50% less pruning cuts than topping cut and 35% less than reduction cut. In both pruning cycles six pruning cuts per plant (42 per treatment) were marked with paint to be recognizable for subsequent measurements. In control trees, six imaginary cuts were drawn on first-order lateral branches, similar in size and age as those used in pruned treatments. Imaginary cuts were drawn next to a lateral having aspect ratio greater than 0.33 compared to the parent branch. In treatments where the apical bud of the branch was retained (i.e. control) or substituted (i.e. reduction), the shoot bearing that apical bud was considered the leader of the branch. In treatments which suppressed the apical bud (i.e. topping and removal cuts), the longest (after the first

134 growing season) of the newly developed sprouts was considered as the new branch leader, while the
135 remaining were considered as laterals (Fig. 2).

136

137 *Biometric measurements, wound closure and breaking stress*

138 In both pruning cycles, all biometric parameters and wound closure were measured at the time of pruning,
139 and 12 and 24 months after pruning. Wound closure was estimated using the woundwood coefficient
140 (Scwharze, 2008), $WC = 100 - [(\pi/4) * b_{t1} * h_{t1} * 100] / [(\pi/2) * (r_{t0})^2]$, where: b_{t1} and h_{t1} are the width and the
141 height of the wound at the time of measurement, and r_{t0} is the initial radius of the pruning wound.

142 Stem diameter was measured at 1.3 m and stem Relative Growth Rate (RGR_{stem}) was calculated as $[\ln(Ø_{t1}) -$
143 $\ln(Ø_{t0})] * (t_1 - t_0)^{-1}$ where: Ø is stem diameter at times 0 and 1, and $t_1 - t_0$ is time (in days) between
144 measurements (Newbery et al., 2011). The number of root suckers developed was counted in each replicate
145 tree. Then, the relative frequencies were calculated, in each treatment, as the ratio between trees releasing a
146 certain number of root suckers (i.e. 0, 1 to 4, 5 to 7, more than 7) over total number of trees of that treatment.
147 Twelve and twenty-four months after each pruning cycle, slenderness of the whole branch ($L:D_{wb}$) was
148 calculated as the ratio between the length and base diameter of pruned branches. Branch length was
149 measured from its attachment to the trunk to the apical bud, while the base diameter was measured at the
150 union with the trunk. Crown dieback was assessed visually 6 and 17 months after each pruning cycle. Crown
151 dieback frequency was calculated, in each replicate tree, as the ratio between pruned branches showing
152 dieback symptoms (i.e. extensive leaf necrosis, absence of growth, bud death) over the total number of
153 marked branches of that treatment. A pruned branch was counted for dieback if showing any of the above
154 mentioned signs on any part of the branch, including sprouts released after pruning.

155 The number, base diameter and length of watersprouts developed or released within 20 cm (as suggested by
156 Grabosky and Gilman, 2007) from the pruning cut or at the callus dieback line were measured 12 and 24
157 months after pruning. Within each marked pruned branch, the slenderness of the leader ($L:D_{leader}$) was
158 calculated as the ratio between the leader length and base diameter. Similarly, the slenderness of the lateral
159 shoots/sprouts ($L:D_{lateral}$) was calculated as the average of the slenderness of all individual sprouts

(excluding, in topping and removal, the sprout designated as new leader) released from the pruning cut. Length of the leader and lateral shoots/sprouts was measured from their attachment on the higher order branch (in most cases, from the pruning cut) to the apical bud, while diameter was measured just above the attachment. The aspect ratio between the lateral and the leader was calculated as the ratio between the base diameter of each lateral shoot and base diameter of the leader.

The stress (σ) required to cause the failure of the attachment between the leader of the branch (or the selected lateral, in control trees) and the parent branch was measured using the methods proposed by Kane et al. (2008). Twenty-four months after each pruning cycle, 14 branch unions per treatment (56 in total in each cycle) were attached to a dynamometer (HCB 200, Kern and Sohn Gmbh, Balingen, Germany), loaded at a rate of 5 cm per minute until breakage of the attachment. Breaking stress (σ) was then calculated as: $32 * P * L * \sin\alpha / (\pi * d^3)$ where: P (kN) is the maximum load; L (m) is the distance between the point of application of the load and the attachment which was kept fixed (about 5 cm); d (m) is the inside-bark branch diameter; α (rad) is the angle between the longitudinal axis of the branch and the applied load.

Leaf gas exchange and integrated leaf anatomical traits

Five and seventeen months after each pruning cycle, after leaves had reached their final size, 10 fully expanded leaves per tree (70 leaves per treatment) were harvested from the leader shoot/sprout of pruned branches and immediately scanned using an A-3 scanner. An image analysis software (Image Tool v1.3, University of Texas, San Antonio, TX, U.S.) was used to measure average leaf area. Leaves were then oven-dried at 70°C until constant weight to determine dry mass. Then, leaf mass per area (LMA) was calculated as the ratio between leaf dry mass and leaf area. Leaf greenness index, a parameter highly correlated to leaf total chlorophyll content in *Acer pseudoplatanus* (R^2=0.9295, see Percival et al., 2008), was measured using a SPAD-meter (SPAD 502. Minolta, Osaka, Japan) on the same leaves used for leaf gas exchange measurements. Leaf gas exchange was measured 4, 5, 6, 15, and 17 months after the first pruning cycle, and 3, 5, 7, 15, and 17 months after the second pruning cycle using an infrared gas analyzer (Ciras 2, PP-System, Amesbury, MA, U.S.). Measurements were conducted between 09.30 A.M. and 12.30 P.M. on the first fully

expanded leaf developed on marked pruned branches (21 leaves per treatment). Leaves in the cuvette were provided with saturating irradiance (1300 μmol m^{-2} s^{-1}, provided using the integrated LED light unit), ambient temperature, relative humidity = 60-80% air, and CO_2 concentration = 380 ppm. Measured parameters were: CO_2 assimilation (A), stomatal conductance (g_s), transpiration (E), and CO_2 concentration in the substomatal chamber (C_i). Instantaneous water use efficiency (WUE) was calculated as A/E. Leaf temperature (T_{leaf}) was measured using the temperature probe integrated in the Ciras cuvette.

CO_2 assimilation was also measured as a function of internal CO_2 concentration (A/C_i curves). A/C_i curves were drawn by decreasing stepwise external CO_2 concentration (C_a) from 380 ppm to 30 ppm, then a C_a of 380 was restored and, finally, C_a was increased stepwise to 1800 ppm (Fini et al., 2014). Curves were drawn 3 and 7 months after the second pruning cycle (May and September, respectively). Estimates of the apparent maximum rate of carboxylation by Rubisco ($V_{c,max}$) and the apparent maximum electron transport rate contributing to ribulose 1,5-BP regeneration (J_{max}) were made from A/C_i curves using the equations found by Sharkey et al. (2007), as described in a previous work (Fini et al., 2011). The stomatal (L_s) and non-stomatal limitations (L_{ns}) to CO_2 assimilation were calculated from A/C_i curves as described in Lawlor (2002) and Long and Bernacchi (2003). Leaf dark respiration was calculated after 20 minutes acclimation to the darkness (provided by switching off the Ciras-2 integrated light source) (Ribas-Carbo et al., 2010). Then, metabolic efficiency of the leaf was calculated as A/R_{dark}.

Statistics

The experimental design was a one-tree per replicate complete randomized design with seven replicates. All data were analyzed with One-Way ANOVA after checking normal distribution of data using the Shapiro-Wilk test (Shapiro and Wilk, 1965). Data which were not normally distributed and parameters with unbalanced samples (i.e. biometrics of watersprouts) were analyzed using the non-parametric Kruskal-Wallis test and means were separated using the Bonferroni test. Frequencies were calculated within each replicate tree and, prior to statistical analysis, were transformed using the formula: arcsin $\sqrt{x}$, where x is the relative frequency (Amoroso et al., 2010). Differences were considered significant at $P < 0.05$ (*) and highly

212 significant at $P < 0.01$ (**). All data were analyzed using SPSS statistical software (SPSS v.20, IBM,
213 NY, U.S.).
214
215 **Results**
216 *Effect on the whole tree and wound closure*
217 Removal cuts yielded larger wounds than topping and reduction cuts, both in the first and in the second
218 pruning cycle (Table 1). Despite being small in size, wounds originated from topping cuts were the slowest
219 in closing and showed little callus and woundwood formation in the two years after pruning.

220 Before the first pruning cycle (February 2008), all trees had similar stem diameter (Table 1). Among the
221 pruning methods investigated, only topping cut depressed stem diameter growth (expressed as stem diameter
222 relative growth rate, RGR_s) compared to control trees (Table 1). RGRs of topped trees was 21% and 34%
223 lower than for control trees after the first and second pruning cycles, respectively. On the contrary, neither
224 reduction nor removal treatments depressed stem growth as compared to control.

225 Presence of dieback on pruned branches increased due to topping (Table 1). In the first pruning cycle, the
226 only treatment to exhibit significant dieback was topping, which displayed dieback on 26% of pruned
227 branches (Table 1). For the second pruning cycle, dieback displayed for topping cuts (37%) and reduction
228 cuts (18%) were 4-fold and 2-fold more frequent than in control branches (9%).

229 70% of topped trees released root suckers, while only 40% of trees pruned with reduction cut, removal cut or
230 left unpruned released root suckers during the growing season after pruning. Furthermore, the frequency of
231 trees with more than 7 root suckers was greatly increased in topped plants compared to all other treatments
232 (Fig. 3A).
233
234 *Effect on the pruned branches: whole branch biometrics and new growth pattern*
235 All pruning techniques reduced the slenderness of the whole branch ($L:D_{wb}$) (Table 2). Removal cut
236 suppresses the whole branch, therefore $L:D_{wb}$ was not measured for this treatment. In the long term (i.e. 24
237 months after pruning), slenderness of topped and reduced branches was similar, despite topped branches

being less slender immediately after pruning (Table 2). During the growing season after pruning, the fast rise of branch slenderness in topped trees was due to the production of very slender watersprouts in response to pruning. Sprouts released after topping cuts were more slender than in all other pruning methods and had similar or even higher slenderness than the unpruned shoots of control trees (Table 2). The increase in slenderness of topped branches was due to increased primary growth, rather than decreased secondary growth (data not shown, but primary growth of the leader shoot was on average 190% and 245% greater than in reduction cut and control, respectively, after 12 months since pruning).

All types of pruning stimulated the release of watersprouts in the proximity of the wound, or directly from the callus (Fig. 3B). In all treatments except topping, however, less than two watersprouts were developed in the 20 cm proximal to the cut in over 80% of pruned branches. On the contrary, over 55% of topped branches released up to 4 watersprouts and over 15% released five or more adventitious sprouts (Fig. 3B). In both pruning cycles, the aspect ratio between the leader and lateral shoots/sprouts within 20 cm from pruning cut was higher in those treatments (removal and topping cuts) which suppressed the apical shoot of the branch without preserving (i.e. control) or substituting (i.e. reduction cut) it (Table 2).

The stress (σ) required to cause the failure of the attachment between the leader and the parent branch was, on average, 64% and 36% lower in topped than in reduced and control branches in the first and in the second pruning cycle, respectively (Table 2). On the contrary, σ in reduction cut and removal cut (the latter measured only in the second cycle) did not differ from control.

Effect on leaf characteristics and gas exchange

Pruning method largely impacted leaf anatomy (Table 3). Leaves developed on topped branches were larger and had lower LMA than in other pruning treatments and in control trees. In the first pruning cycle, the effect of topping on leaf size and LMA was significant in the first growing season after pruning, but not in the second one. As pruning was repeated, the effect of pruning method on leaf size and LMA lasted longer, and leaves developed on topped branches still had larger leaf area and lower LMA even in the second growing season after pruning. Leaves originated on topped branches were about 1 °C warmer than leaves of control branches during the late-spring and summer period (data are the average of three measurement days

conducted from May to September) (Table 3). On the contrary, neither removal nor reduction treatments lead to significant leaf warming compared to control.

Only topping cut increased significantly the leaf greenness index (Table 3). The effect was indeed transitory, being only significant in the growing season immediately after pruning, then disappearing or being greatly reduced the following season. Similarly, the apparent carboxylation efficiency by Rubisco ($V_{c,max}$) and the apparent contribution of electron transport to Ribulose regeneration (J_{max}) were higher in the topping treatment than in control during the first growing season after pruning (Table 3). Significant difference in $V_{c,max}$ and J_{max} were found between these two treatments both in May and in September, 3 and 7 months after pruning, respectively. On the contrary, leaves developed on reduced or removed branches had similar $V_{c,max}$ and J_{max} to control.

The effects of pruning method on CO_2 assimilation (A) were mostly restricted to the first few months following pruning (i.e. 3 and 4 months after the second and the first pruning cycle, respectively) (Fig. 4A). Early after pruning, only leaves of topped branches displayed higher A than control trees in both pruning cycles. Later on during the growing season, differences among treatments disappeared, except on late summer 2011 (17 months after the second pruning cycle), when the removal treatment displayed lower A than the reduction and control treatments. Stomatal conductance (g_s) was not affected by pruning method in the first pruning cycle (Fig. 4B). When pruning was repeated, an early enhancement of g_s was observed 3 months after pruning in the topping and reduction treatments compared to removal and control (Fig. 4B). Later on in the growing season (i.e. 5 and 7 months after 2nd pruning cycle, July and September respectively), g_s decreased in topped trees and increased in control, making the differences in g_s less substantial. In the second growing season after the 2nd pruning cycle, leaves held on branches developed after the removal cut had lower g_s than leaves of the other treatments. Intercellular CO_2 concentration (C_i) was generally decreased by topping and removal cuts in the first summer after the first pruning cycle (5 and 7 months after pruning), then differences were not confirmed in the second growing season (15 and 17 months after pruning) (Fig. 4C). As pruning was repeated, the lower C_i during summer in leaves of the topping treatment, compared to control, was confirmed (5 and 7 months after the second pruning cycle), and differences were still significant in the second growing season (15 and 17 months after pruning) (Fig. 4C).

Three months after pruning, early after full leaf expansion (May 2010), all types of pruning reduced stomatal limitation to CO_2 assimilation (L_s) compared to control (Table 4). Similarly, non-stomatal limitation (including mesophyll diffusion and biochemical limitations) (L_{ns}) were lower in pruned than in control leaves. In detail, topping cut lead to the largest decrease in L_{ns}, whereas removal cut the least (Table 4). As season progressed, and trees had to cope with stresses such as heat and reduced water availability (see the change in air temperature and rainfall from May to July 2010 in fig. 1), L_s increased to a greater extent in topping than in removal and reduction treatments, while control showed the lowest increase (Table 4, 7 months after pruning). Similarly, L_{ns} increased in all pruned treatments, but particularly in leaves of topped trees which, however, yet displayed lower L_{ns} than control trees, as shown by the negative L_{ns} value.

Discussion

Shigo (1989) described pruning as "the best thing an arborist can do for a tree but at the same time, one of the worse things an arborist can do to a tree; much depends on how pruning is carried out". Results of this experiment support Shigo's statement by providing a quantitative evaluation of the effects of different pruning methods, scaling down from the whole tree to leaf physiology and biochemistry.

Pruning treatments mainly differed because the apical bud of the pruned branch was suppressed (topping), substituted (reduction) or retained (control), while removal cut suppressed the whole primary branch, instead of its apical portion. We hypothesized that these methods may differently disturb apical dominance thereby affecting subsequent growth and physiological processes and, in particular, that substituting the apical bud of the branch with the one of a properly sized lateral branch through reduction cut may, at least in part, avoid the complete release of apical dominance which occurs after chopping off (i.e. topping) (Hillman, 1984). Results of this study clearly confirm this hypothesis.

First, only topped trees showed reduced stem diameter growth and increased release of root suckers compared to controls. Reduction in stem diameter growth have been reported for intense pruning treatments (i.e. > 50% leaf area, Pinkard and Beadle, 1998; Neilsen and Pinkard, 2003), but were unexpected here, as only 30% of tree canopy was removed (Maurin and DesRochers, 2013). Because the amount of leaf area removed by all pruning treatments was similar, it is unlikely that diminished stem growth of topped trees is

due to reduced availability of photosynthetates. Unlike other pruning methods, topping cut most likely triggered the change in biomass partitioning to favour neoformed sprouts, at least partly at expenses of stem growth, as reported to occur in severely defoliated trees (Hoogesteger and Karlsson, 1992; Pinkard and Beadle, 1998). This is consistent with the higher emission of root suckers and watersprouts observed in topped than in control trees. Enhanced release of sprouts from lateral, adventitious and latent bud has been related to suppressed apical dominance (Cline, 1997). Consistently, our data show that while topping had a severe effect on tree structure by greatly suppressing apical control and promoting epicormic growth, pruning back a branch to a lateral with intact apical bud and large enough to become the new branch leader (i.e. reduction cut) preserved normal tree growth pattern (Wilson, 2000). Similarly, removal of the whole branch at its attachment to the trunk resulted in minimal disturbance to tree structure. In fact, epicormic sprouts developed next to the removal pruning cut grew in the inner part of the crown (particularly after the 2nd pruning cycle, when trees were larger) under reduced light availability, which greatly limit their primary growth and slenderness (Solomon and Blum, 1977). This is probably due to lower sink strength than the stem they are attached to, resulting in photoassimilates export from the sprouts (Stoll and Schmid, 1998; Wilson, 2000). One of major disturbances of pruning to tree structural strength is that it inextricably leads to open wounds, which may be a preferential point of entry for wood decay fungi. In this experiment, removal cuts yielded larger wounds than all other treatments but, contrary to the previously reported inverse relation between wound size and time of closure (Solomon and Shigo, 1976), wounds from removal cuts were the fastest at closing. Not only wound size, but also the wound location within a tree, can affect wound closure process. Larger wounds have been shown to lead to greater amount of discoloured wood, while poor correlations are generally found between the amount of discoloured wood and closure time (Solomon and Shigo, 1976; Gilman and Grabosky, 2007). It was shown, however, that the amount of wood discoloration is inversely related to the vigour of the wounded plant organ, and that wound closure time is also inversely related to vigour (Solomon and Blum, 1977; Armstrong et al., 1981).

Second, on the pruned branches, codominance of newly developed sprouts was triggered in treatments which suppressed the apical axis or the whole branch, without providing a new leader. In fact, new sprouts (branches in the following year) developed on topped branches and after branch removal had aspect ratio higher than 0.7 whereas aspect ratios lower than 0.5 were found in reduced branches and control, as normally

occurs between leader and subordinate branches (Grabosky and Gilman, 2007; Gilman, 2012). We show here that if the apical branch is substituted by a properly sized lateral, the latter has enough sink strength to prevent extensive outgrowth from lateral buds and to maintain apical control over subodrdinate laterals, indicating that reduction cut achieves in maintaining apical control whereas topping cut does not. From a management viewpoint, codominance is one of the most hazardous structural defects of a tree which usually leads to reduced tree safety, particularly if codominant branches are slender and weakly attached to the trunk or the parent branch (Dahle et al., 2006; Gilman, 2012; Ciftci et al., 2013). Indeed, the stress required to break the union between newly developed sprouts and their parent topped branch was about 1/3 to 2/3 lower than that required to tear apart a normal branch union. This corroborates the idea that, in topped branches, most of regrowth occurs from adventitious buds, which are inextricably weakly attached to the parent branch, since they are attached at the cambium level (Dahle et al., 2006). Also, sprouts released in topped branches were more slender than in other pruning treatments and, after the second pruning cycle, the leader sprouts of topped branches were even more slender than unpruned shoots in control plants, although the same was not observed for lateral sprouts. High slenderness may not be an issue for young growing axis, which are flexible enough to avoid fractures even at high wind loads, which may instead cause the failure of the attachment, particularly if the branch union is weak (Bertram, 1989). As branches grow old and increase in size, switching from a "light-harvesting" to a structural role (which occurs when the branch is about 3 m in length) slenderness starts to decline (Bertram, 1989; Dahle and Grabosky, 2010), because of reduced elongation, rather than to smaller diameter growth (Dahle and Grabosky, 2010). Topping cut hinders this normal ageing process of the branch by stimulating primary branch growth (long about 2.7 m just before the second pruning cycle) and prevents the branch from performing a structural role. Pioneer works hypothesized that removal of the apical axis may stimulate lateral axis to elongate more than they would have done if the terminal had remained intact (Wilson, 1990), because of altered hormonal balance (Thimann and Skoog, 1934; Prochazka and Jacobs, 1984), demonstrating that all lateral shoots have the potential to become long shoots if not dominated (Suzuki and Kohno, 1987). The effects of reduction, removal and topping cuts on tree hormones was not tested in this experiment; however, this knowledge would be of great importance for determining best pruning practices and deserves to be addressed by future research. The fast growth rate (in length) of pruned branches, the increased codominant branching and the weak branch

attachments in topped branches out-compassed the safety benefit resulting from the initial greater reduction of whole branch slenderness immediately after pruning. Thus, despite topping appearing as a cheap and fast pruning method in the short term, it has deleterious mid- and long-term effects on tree structure, thereby resulting in the need of more frequent pruning and in a 4-fold rise of overall pruning cost (Campanella et al., 2009).

Third, from the physiological viewpoint, topping stimulated vigorous resprouting from pruned branches, but at the expenses of stem diameter growth and of the capacity to withstand unfavourable conditions in the long-term (Harris et al., 2004; Spann et al., 2008). This may be due to the enhanced competition for light and nutrients among watersprouts released from the same pruning cut. After apical control is removed by a properly executed cut, the distal branch grows larger and more vertical, replaces the removed terminal and restores apical control (Wilson, 2000). On the contrary, topping cut releases several co-dominant sprouts all located close to the wound without any distal shoot. In this situation, becoming larger and developing larger leaf area provides competitive advantage, because of higher hormone production and greater light harvesting capacity compared to shorter sprouts with smaller leaf area (Wilson, 2000). Growth rate greatly depends on leaf structural, biochemical and functional characteristics, with leaves with small LMA and high nitrogen and chlorophyll content being commonly associated with fast-growing strategies (Reich et al., 1992: Poorter and Bongers, 2006). Among pruning treatments tested here, only topping affected leaf structural traits such as leaf area and leaf mass per area. The larger area of individual leaves of topped branches increased the photosynthetic surface of individual branches, but resulted in leaf over-heating because of lower heat dissipation by conduction/convection, than smaller leaves (Nobel, 2005). Increased leaf area in topped trees was paralleled by a decrease in leaf mass per area. Leaves with low LMA are productive and often associated with fast-growing plant strategies, but are necessarily short-lived and more susceptible to environmental stresses (Wilson et al., 1999; Bussotti, 2008; Poorter et al., 2009). Consistently, a greater occurrence of crown dieback was observed in topped trees than in other treatments after both pruning cycles.

Transient (lasting few weeks to few months) increases in net CO_2 assimilation (compensatory photosynthesis) have been reported to occur following pruning and partial defoliation, with the magnitude of this increase being positively correlated with pruning/defoliation severity (Pinkard et al., 1998; Hart et al.,

2000; Turnbull et al., 2007). We show here that the type of pruning, not only its severity, can modulate tree responses at the leaf level. Mechanisms leading to compensatory photosynthesis are still poorly understood and may involve increased stomatal conductance, increased leaf nitrogen and chlorophyll, and increase $V_{c,max}$ and J_{max} (Sharkey, 1985; Pinkard et al., 1998; Pinkard and Beadle, 1998; Turnbull et al., 2007). Leaf structure is generally optimized for maintaining the operating [CO_2] in the chloroplast stroma (C_c) at the transition between the Rubisco carboxylation and RuBP regeneration limitations to photosynthesis (Farquhar et al., 1980), to reduce photorespiration and, consequently, increase CO_2 assimilation (Terashima et al., 2011). A tight co-regulation of stomatal and non-stomatal factors is required to achieve this goal (Flexas and Medrano, 2003). Topping cut lead to an imbalance of stomatal regulation (when compared to control) which was not observed in other pruning treatments. In fact, non-stomatal limitations to photosynthesis were much lower in topping than in other pruning treatments and than in control, because Rubisco activity and the contribution of electron transport to ribulose regeneration were greatly up-regulated in leaves developed in topped branches. Higher $V_{c,max}$ and J_{max} in topping treatment resulted in transient increases in net CO_2 assimilation, when stomatal conductance was high enough to maintain adequate leaf internal CO_2. Later in the growing season, however, stomatal limitations increased more in leaves of topped plants than in other treatments. Although leaves on topped branches still showed higher $V_{c,max}$ and J_{max} after the summer period (7 months after 2nd pruning cycle), they did not show enhanced CO_2 assimilation rate compared to other treatments because of high stomatal limitations, as previously hypothesized (Pinkard et al. 1998; Pinkard and Beadle 1998). Maintaining higher $V_{c,max}$ and J_{max} requires large complements of enzymes and other metabolites which have a substantial maintenance cost and require periodic (and costly) recycling (Reich et al., 1998). Moreover, higher leaf chlorophyll and nitrogen content are commonly associated with higher respiration rates (Reich et al., 1998). Consistently, the A/R_d ratio was significantly lower in leaves on topped branches than in other treatments as soon as CO_2 assimilation declined because of stomatal limitation, indicating that metabolic inefficiency at the leaf level is promoted by topping (Cai et al., 2009).

The morpho-physiological changes induced by topping were not found in plants pruned by the reduction cut, suggesting that apical dominance and control may be effectively retained if the branch is pruned to a lateral, large enough to become the new dominant primary axis. Removal cut, similar to topping cut, removes the primary axis without proving a substitution leader. However, disturbance to plant physiology was much

lower, as watersprouts developed from pruning cut grow in the inner part of the canopy, and self-shading resulted in a generally low photosynthetic rate, stomatal conductance and, presumably sink strength (McCormick et al., 2006). This effect was clear particularly after 2nd pruning cycle, when plants were larger and with broader and denser canopies, which resulted in a denser shade cast on new shoot developing from the trunk.

In conclusion, we show here that pruning method, not only its severity, modulates the morpho-physiological response of trees to pruning. Maintenance of apical control and apical dominance are key issues to preserve a structurally sound tree structure, as well as the long-term efficiency of the photosynthetic apparatus. While removal of the whole primary axis at its attachment to the trunk provide minimal disturbance to tree physiology, shortening of the branch may provide different results, depending on where the branch is shortened. Reducing the primary axis to a lateral branch large enough to become the new branch leader appeared to preserve normal branching pattern and had little effects on leaf structure and photosynthetic performance. On the contrary topping a branch (shortening of the primary axis without providing a substitution leader) greatly affected tree structure and functioning by altering branching pattern, by promoting competition among sprouts of the same branch, and by determining a shift toward a more pioneer (fast growing) behaviour, but at the expense of tolerance to environmental stresses. It must be noted, however, that this work dealt with young trees and further research is needed to evaluate the physiological response to pruning method in mature or senescing trees.

Acknowledgements

This work has been done as a part of a research project called “Miglioramento delle tecniche produttive e della qualità del prodotto nel vivaismo ornamentale - TECPRO” financed by Regione Lombardia – Agricultural Department, according to the Plan of Research and Development 2008. Partial funding was provided by UNISER Consortium, Pistoia (Italy)

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634 **Figure captions**

635 Figure 1: Monthly average temperature (°C) and rainfall (mm) at the experimental site (Vertemate con
636 Minoprio, CO, Italy, 45° 44' N, 9° 04' E, 250 m above sea level) during the experimental period (2008 –
637 2011).

638 Figure 2: Schematic diagram of the pruning treatments imposed and of the effects of the different pruning
639 methods on new growth. The black triangles indicate that the apical bud of the branch was left untouched.
640 "Leader" and "laterals" indicate which shoots/sprouts were considered as dominant and subordinate growing
641 axes, respectively.

642 Figure 3: Frequency of: (A) number of root suckers released from the trunk flare, and (B) number of
643 watersprouts released within 20 cm from pruning cut during the first growing season after the first pruning
644 cycle. Frequencies were calculated on 7 replicate trees per treatment (root suckers) or 42 replicate pruning
645 cut per treatment (watersprouts). Different letters within the same frequency class indicate significant
646 differences among treatments at $P < 0.05$ (*) or $P < 0.01$ (**).

647 Figure 4: Effect of different pruning treatments on: A) CO_2 assimilation (A, μmol m^{-2} s^{-1}); B) stomatal
648 conductance (g_s, mmol m^{-2} s^{-1}); and C) CO_2 concentration in the substomatal chamber (C_i, ppm) measured in
649 the 17 months after the first and the second pruning cycle. Different letter within each sampling date indicate
650 significant difference among treatments at $P < 0.05$ (*) or $P < 0.01$ (**).

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653 Table 1: Effect of different pruning methods on wound size (cm^2) and wound closure (estimated using the
654 woundwood coefficient, see method section for details), on stem relative growth rate (RGR, μm cm^{-1} d^{-1}) and
655 on the frequency of dieback on pruned branches after the first and the second pruning cycle. Stem diameter
656 measured in February 2008, right before the first pruning cycle, is also reported. Different letters within the
657 same row denote significant differences among pruning treatments at $P < 0.01$. n.d. = not determined.

	Pruning cycle	Months after pruning	Topping	Reduction	Removal	Control	P
Wound size (cm^2)	1	0	2.5 b	2.7 b	4.2 a	n.d.	0.000
	2	0	3.3 b	4.1 b	7.1 a	n.d.	0.000

Woundwood coefficient (%)	1	12	0 b	65 a	44 b	n.d.	0.000
	1	24	10 b	93 a	72 a	n.d.	0.000
	2	12	4 b	17 a	19 a	n.d.	0.000
	2	24	24 b	43 a	50 a	n.d.	0.000
Stem diameter (cm)	1	0	6.1	6.2	6.6	6.3	0.232
RGR_{stem} ($\mu m\ cm^{-1}\ d^{-1}$)	1	0-24	8.1 b	10.8 a	10.0 a	10.3 a	0.003
	2	0-24	6.2 b	8.5 a	8.8 a	9.4 a	0.001
Crown dieback (%)	1	17	26 a	0 b	3 b	0 b	0.008
	2	17	37 a	18 b	6 c	9 c	0.005

Table 2: Effects of different pruning methods on branch biometrics: slenderness of the whole branch ($L:D_{wb}$, cm cm^{-1}); aspect ratio between the dominant and the subordinate shoots within 20 cm from pruning cut (cm cm^{-1}); slenderness of the dominant shoot/sprout of the branch ($L:D_{leader}$, cm cm^{-1}) and of subordinate shoots/sprouts ($L:D_{lateral}$, cm cm^{-1}) and stress required to cause the failure of the union between the dominant shoot of the branch and the parent branch (σ, MPa). Different letters within the same row denote significant differences among pruning treatments. n.d. = not determined

	Pruning cycle	Months after pruning	Topping	Reduction	Removal	Control	P
$L:D_{wb}$ (cm cm^{-1})	1	0	24.2 c	35.4 b	n.d.	63.7 a	0.000
	1	12	58.5 b	64.6 b	n.d.	81.5 a	0.000
	1	24	75.8 b	75.9 b	n.d.	85.9 a	0.004
	2	0	18.3 c	57.2 b	n.d.	88.4 a	0.000
	2	12	46.8 c	64.4 b	n.d.	89.1 a	0.000
	2	24	69.9 b	71.0 b	n.d.	89.1 a	0.002
aspect ratio (cm cm^{-1})	1	12	0.86 a	0.34 b	0.82 a	0.31 b	0.000
	1	24	0.80 a	0.34 b	0.75 a	0.41 b	0.008
	2	12	0.77 a	0.29 c	0.91 a	0.47 b	0.004
	2	24	0.78 a	0.30 b	0.73 a	0.46 b	0.009
$L:D_{leader}$ (cm cm^{-1})	1	24	94.2 a	79.4 b	60.5 c	89.9 a	0.008
	2	24	95.2 a	80.6 b	60.3 c	75.5 b	0.000
$L:D_{lateral}$ (cm cm^{-1})	1	24	84.6 a	79.3 b	52.3 c	80.9 ab	0.015
	2	24	89.9 a	71.2 b	51.3 c	86.9 a	0.000
σ (MPa)	1	24	20.1 b	47.0 a	n.d.	53.7 a	0.020
	2	24	37.4 b	62.4 a	47.4 ab	58.5 a	0.029

Table 3: Effects of different pruning methods on leaf morpho-physiological characteristics: average leaf area (cm^2), leaf mass per area (LMA, g m^{-2}), leaf temperature (T_{leaf}, °C), leaf greenness index, apparent rate of carboxylation by Rubisco ($V_{c,\ max}$, μmol $m^{-2}\ s^{-1}$); apparent contribution of electron transport to ribulose regeneration (J_{max}, μmol $m^{-2}\ s^{-1}$), and ratio between net CO_2 assimilation and dark respiration (A/R_{dark}). Different letters within the same row denote significant differences among pruning treatments at $P < 0.05$.

	Pruning	Months after	Topping	Reduction	Removal	Control	P

	cycle	pruning					
Leaf area (cm^2)	1	5	270.8 a	199.0 b	188.0 b	220.8 b	0.000
	1	17	210.1	166.2	152.5	172.1	0.683
	2	5	279.9 a	165.22 b	155.3 b	147.8 b	0.010
	2	17	183.5 a	165.9 b	155.9 b	131.6 c	0.000
LMA ($g\ m^{-2}$)	1	5	80.1 b	98.9 a	99.9 a	93.1 a	0.038
	1	17	87.3	93.3	97.8	93.8	0.817
	2	5	88.6 c	106.5 a	96.2 b	104.5 ab	0.016
	2	17	78.5 b	95.8 a	93.7 a	94.4 a	0.003
T_{leaf} (° C)	1	4-6	30.5 a	29.6 b	29.3 b	29.4 b	0.000
	1	15-17	29.7 a	29.3 b	29.0 b	29.2 b	0.000
	2	3-8	31.0 a	30.1 b	30.0 b	29.9 b	0.000
	2	15-17	29.3 a	28.4 b	28.6 b	28.3 b	0.000
Leaf greenness index (a.u.)	1	3-8	45.0 a	42.9 b	39.0 c	40.2 bc	0.000
	1	15-17	40.0	40.9	39.6	40.1	0.482
	2	3-8	42.7 a	39.1 b	35.0 c	36.9 bc	0.000
	2	15-17	39.6 a	38.3 a	35.7 b	37.3 ab	0.005
$V_{c,max}$ ($\mu mol\ m^{-2}\ s^{-1}$)	2	3	124.0 a	103.2 ab	93.4 b	89.5 b	0.030
	2	7	133.6 a	98.0b	93.1 b	96.0 b	0.000
J_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	2	3	226.3 a	165.5 b	141.4 b	130.2 b	0.001
	2	7	198.0 a	156.0 b	152.6 b	146.3 b	0.000
A/R_{dark}	2	3	13.06	14.34	14.11	13.08	0.754
	2	7	8.91 b	13.57 a	13.72 a	12.43 a	0.000

Table 4: Stomatal (L_s) and non-stomatal (L_{ns}) limitations to photosynthesis in leaves of *A. pseudoplatanus* developed on branches subjected to different pruning methods. L_{ns} was calculated as in Lawlor (2002) and Long and Bernacchi (2003) using control leaves as reference parameter. Negative L_{ns} indicates lower non-stomatal limitations to CO_2 assimilation than in control trees. Different letters within the same row denote significant differences among pruning treatments at $P < 0.05$.

	Pruning cycle	Months after pruning	Topping	Reduction	Removal	Control	P
L_s (%)	2	3	9.8 b	9.9 b	10.9 b	16.6 a	0.035
	2	7	41.0 a	21.9 b	21.9 b	18.8 b	0.015
L_{ns} (%)	2	3	-52.3 c	-16.6 b	-2.7 a	-	0.039
	2	7	-25.4 b	5.4 a	4.4 a	-	0.012

- Pruning method, not only its intensity, modulates the tree response to pruning
- Reducing the apical growing axis to a lateral little disturbs branch growth
- Topping increases codominance and weakens branch structure
- In topping, higher $V_{c,max}$ and J_{max} are not paralleled by higher CO_2 assimilation

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Figure 1

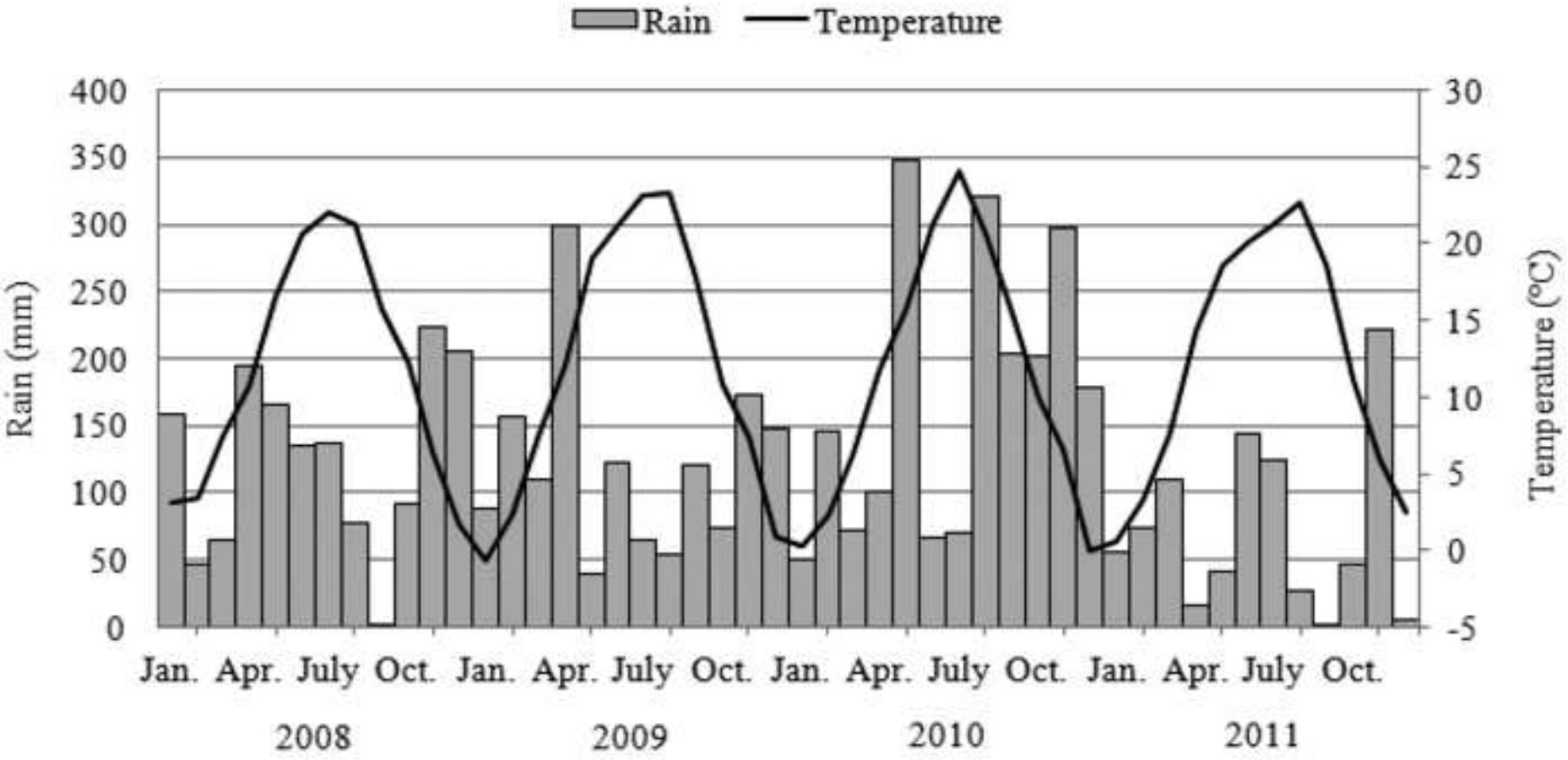
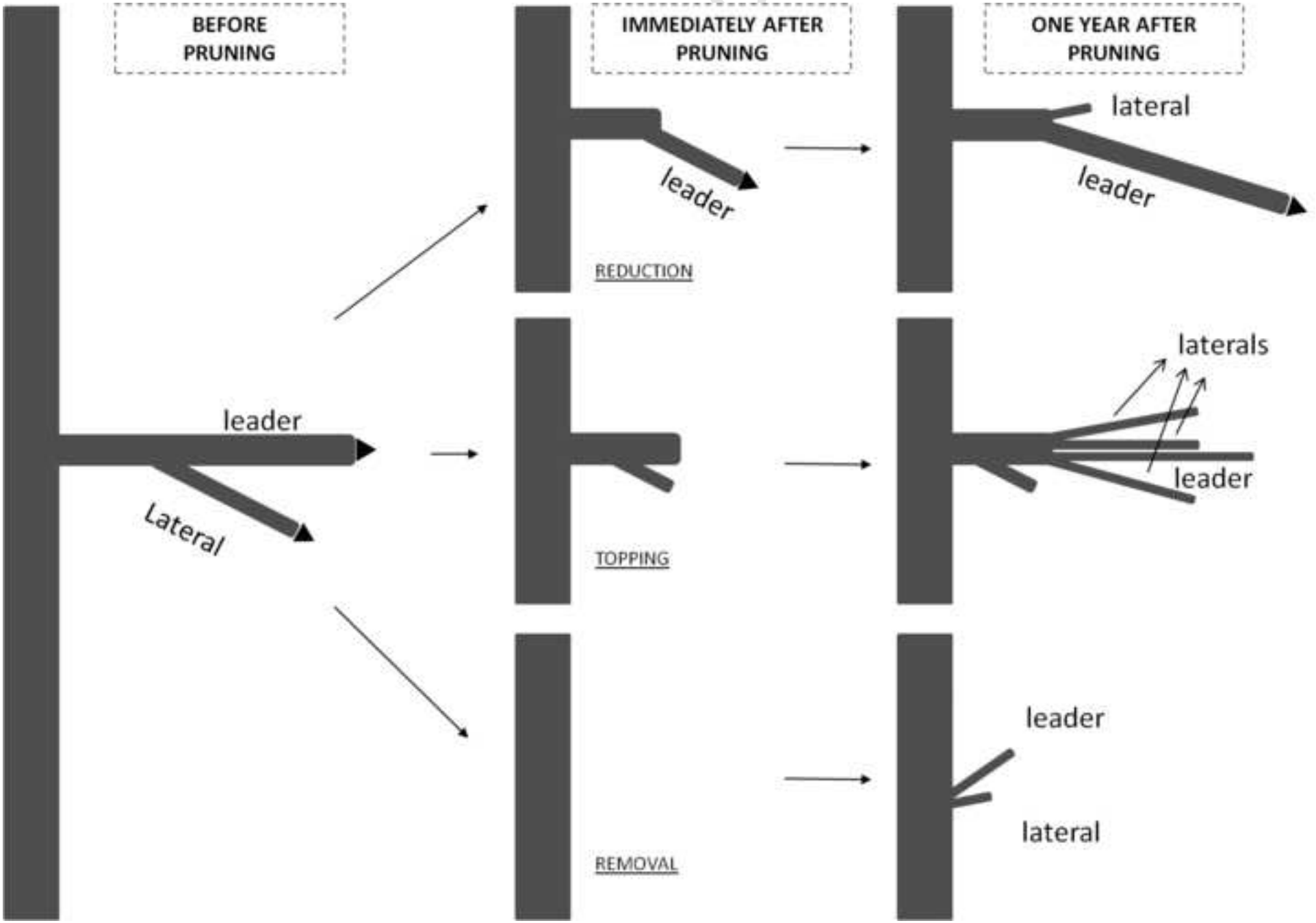

Rain
Temperature
Rain (mm)
Temperature (°C)
400
350
300
250
200
150
100
50
0
30
25
20
15
10
5
0
-5
Jan. Apr. July Oct. Jan. Apr. July Oct. Jan. Apr. July Oct. Jan. Apr. July Oct.
2008
2009
2010
2011

Figure 2


BEFORE PRUNING
IMMEDIATELY AFTER PRUNING
ONE YEAR AFTER PRUNING
leader
Lateral
leader
REDUCTION
lateral
leader
TOPPING
laterals
leader
REMOVAL
leader
lateral